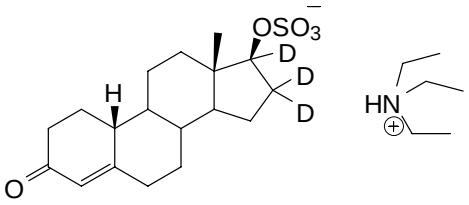
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D782b.2012.02 (Ampouled 090107)
This batch of ampoules was prepared from the bulk material on 7th January 2009.

Compound Name: **d_3-Nandrolone sulfate (triethylammonium salt)**
Description: Off white solid
Collection Number: D782b
Batch Number: 08-S-01
Chemical Formula: $C_{24}H_{38}D_3NO_5S$
Molecular Weight: 458.7
CAS Number: N/A
Batch Production Completed: August 2008
Structure:



Synonyms: (16,16,17α-d_3)-Nandrolone sulfate, triethylammonium salt
d_3-17β-sulfooxy-4-estren-3-one triethylammonium salt
d_3-estr-4-en-17β-ol-3-one triethylammonium salt

The main component of this material is d_3-nandrolone sulfate as a combination of the triethylammonium salt (~78%) and as the protonated sulfate (~22%). d_2-, d_1- and d_0-Nandrolone sulfate are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_3, d_2 and d_1) and d_0- nandrolone sulfate anion in the material.

The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D782b. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 720 µg of anhydrous nandrolone sulfate anion (d_3, d_2, d_1 and d_0).

This material should be considered for use as an internal standard only.

Isotopic Purity: $d_3 \approx 92\%$ $\left[= \frac{d_3}{d_3 + d_2 + d_1 + d_0} \times 100 \right]$

$d_0 < 0.5\%$ $\left[= \frac{d_0}{d_3} \times 100 \right]$

(determined by SIM analysis of d_3-nandrolone used to prepare D782b)

HPLC:
Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltima C-18, 5 µm (4.6 mm × 150 mm)
Column temperature: 40 °C
Mobile Phase: Solvent A: 10 mM ammonium acetate
Solvent B: acetonitrile
Gradient 0-5 min 75% A, 10-18 min 40% A, 20-26 min 75% A
Flow Rate: 1.0 mL/min
Waters PDA 996 operating at 242 nm (2009)
Detector: Waters PDA 2998 operating at 242 nm (2012)
Retention time: 9.9 min (2009), 9.3 min (2012)
Relative peak area response of main component:
Initial analysis: Mean = 95.97%, s = 0.18% (7 ampoules in duplicate, January 2009)
Re-analysis: Mean = 90.1%, s = 0.5% (5 ampoules in duplicate, February 2012)

Detector: Waters ELSD 2420
Retention time: 9.9 min
Relative peak area response of main component:
Initial analysis: Mean = 92.8%, s = 0.75% (7 ampoules in duplicate, January 2009)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The purity value was obtained from traditional analytical techniques. The purity estimate by traditional analytical techniques was obtained by subtraction from 100 % of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Solvent A: 10 mM ammonium acetate Solvent B: acetonitrile Gradient 0-5 min 75% A, 10-18 min 40% A, 20-26 min 75% A
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 at 254 nm
	Retention time:	10.14 min
	Relative peak area response of main component:	
	Initial analysis:	Mean = 96.6%, s = 0.06% (10 sub samples in duplicate, May 2008)
ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Negative ion mode, direct infusion at 5 µL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	650 V
	Cone voltage:	30 V
	Peak:	356 (M-Et_3NH^+) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (17/3) Single spot observed, R_f =0.32. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3034, 2909, 2518, 2361, 1680, 1622, 1472, 1260, 1196, 602 cm^{-1}
^{1}H NMR:	Instrument:	DMX 600
	Field strength:	600 MHz Solvent: $CDCl_3$
	Spectral data:	δ 0.85 (3H, s), 0.8-0.9 (1H, m), 1.0-1.1 (2H, m), 1.2-1.4 (4H, m) 1.41 (“6.7” H, t, *J* = 7.3 Hz), 1.51 (1H, m), 1.62 (1H, dd, *J* = 7.3, 12.3 Hz), 1.80 (2H, m), 1.97 (1H, m), 2.07 (1H, m), 2.2-2.3 (3H, m), 2.40 (1H, m), 2.48 (1H, dd, *J* = 1.5, 7.9 Hz), 3.05 (“4.7”H, t, *J* = 7.3 Hz) 5.83 (1H, s) ppm
^{13}C NMR:	Instrument:	DMX 600
	Field strength:	150 MHz Solvent: d_4-MeOH (48.0 ppm)
	Spectral data:	δ 10.6, 11.1, 22.9, 26.1, 26.7, 30.9, 35.5, 36.3, 36.7, 40.4, 42.6, 42.7, 42.9, 49.5, 50.0, 123.8, 169.8, 201.9 ppm
Thermogravimetric analysis:		Initial volatile content < 0.1% and non volatile residue < 0.2%mass fraction (July 2008)
Karl Fischer analysis:		Moisture content 0.3% mass fraction (February 2008)

Expiration of certification
The property values are valid till 29th February 2015, i.e. three years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body.

The expiry date/shelf life does not apply to ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

The long-term stability of the compound in solution has not been examined.

In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years.

Recommended storage
When not in use this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

Intended Use
For ***in vitro*** laboratory analysis only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 17 July, 2012.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 17th July 2012.